DELETIONS AND FUNCTIONS OF THE CENTER OF THE φ80-λ PHAGE GENOME. EVIDENCE FOR A PHAGE FUNCTION PROMOTING GENETIC RECOMBINATION[1]

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THE genetic map of vegetative λ phage consists of three regions: a right arm defining primarily "early" functions, a left arm responsible for "late" functions, and a center (JACOB, FUERST and WOLLMAN 1957; DOVE 1966; EISEN *et al.* 1966; JOYNER, ISSACS and ECHOLS 1966; SKALKA 1966) (Figure 1a). Genetic recombination occurs with roughly equal frequency in each of these regions (KAISER 1955; AMATI and MESELSON 1965). Yet despite extensive search for mutations in essential phage functions (CAMPBELL 1961), none required for vegetative development has been assigned to the center region. This would allow the possibility that the genetic distance represented there might not be correlated with physical length of DNA. However, deletions of sizable amounts of center DNA have been observed (KELLENBERGER, ZICHICHI and WEIGLE 1961), and the results reported here affirm that at least 30% of lambda's DNA can be deleted from the center region; in none of these instances is there loss of any function essential to the replication and maturation of vegetative phage. On the other hand, we do find correlated with the deletion of center DNA losses of an interesting group of functions nonessential to vegetative development, perhaps all related to processes involving DNA as substrate.
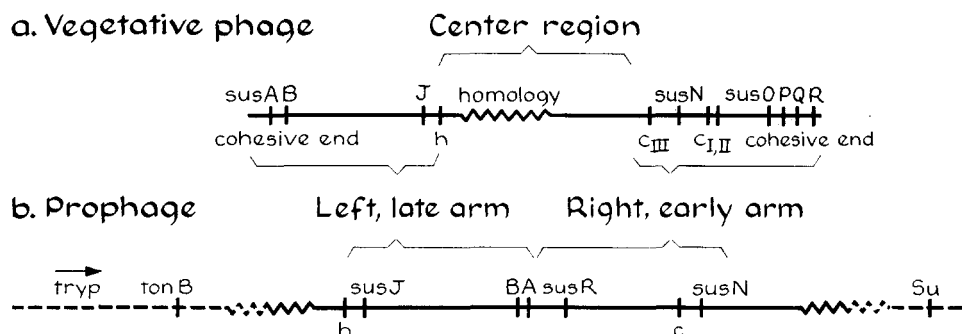

FIGURE 1.—Genetic maps of (a) vegetative phage and (b) prophage, drawn approximately to scale. Phage genome is indicated by solid line, bacterial by broken line, homology by zigzag line. The markers given for the vegetative phage are from among those known for λ (CAMPBELL, 1961; EISEN *et al.* 1966; KAISER 1957). The markers given for prophage are those known for *i*φ80*h*+λ (FRANKLIN *et al.* 1965), shown integrated at the φ80 attachment site. The two phages are believed to have analogous genomes.

Bacteriophages: $i^{\phi 80}h^{+\lambda}$: a hybrid between temperate phages λ and $\phi 80$ (FRANKIN *et al.* 1965). Various mutations of this virus are described in the text.

$i\lambda cI857h^{+\phi 80}$: a hybrid between $\phi 80$ and heat-inducible (SUSSMAN and JACOB 1962) $\lambda cI857$.

$\lambda b2$: a low density mutant of λ. The standard λ and $\lambda b2$ used here were shown to have the same relative positions after equilibrium sedimentation in CsCl as was previously described (KELLENBERGER, ZICHICHI and WEIGLE 1961).

sus mutants (suppressor sensitive) are unable to grow in non-permissive bacteria, but able to grow in permissive bacteria. They are designated by capital letters, which stand for different cistrons (CAMPBELL 1961).

Phage lysates were prepared in soft-agar layer on broth plates.

Bacteria: All *Escherichia coli* K-12.

W1485: permissive, universal host. Also lysogens derived from W1485.

W3101: non-permissive.

CR63: permissive, λ-resistant, λh-sensitive.

W3101/λ: non-permissive, λ-resistant, λh-sensitive.

AB2463, AB2470, Ab2462, JC4451: recombination-deficient (Rec$^-$) mutants (HOWARD-FLANDERS and THERIOT 1966; J. CLARK, personal communication) of AB1157, nonpermissive for the phage *sus* mutants used here.

Bacteria were grown to log phase in broth, centrifuged and washed once in 10^{-3} M MgSO$_4$ in preparation for adsorption of phages.

Medium: broth: 5 g NaCl, 10 g Bacto tryptone, 1 liter H$_2$O; 12.5 g Bacto agar added for plates; 7 g Bacto agar added for soft-agar top layer.

CsCl density gradient centrifugation: Phage suspensions were mixed with an equal volume of Tris-buffered (pH 7.3) saturated CsCl (to give refractive index 1.3800), and centrifuged in the Spinco SW 50 swinging bucket roter at 29,000 rpm for 20 hours at 20°C. The polyallomer centrifuge tubes were then pierced at the bottom to allow serial collection of the contents by drops. The phage bands were always at least 40 drops distant from top and bottom of the gradient.

RESULTS

Low-density mutants of $i^{\phi 80}h^{+\lambda}$: The phage deletion mutants used here were generated in a new way, starting from a defective prophage. Originally the prophage was a nondefective hybrid between related phages λ and $\phi 80$, having the immunity of $\phi 80$ ($i^{\phi 80}$) and the host range of wild-type λ ($h^{+\lambda}$), and integrating into the chromosome of *E. coli* at the $\phi 80$ attachment site (FRANKLIN, DOVE and YANOFSKY 1965). The latter lies adjacent to the *tryp* operon and gene *tonB* (genetic symbols given in DEMEREC *et al.* 1966), which determines sensitivity to phage T1 (Figure 1b). The selection of spontaneous T1-resistant mutants from this lysogen led to the recovery of strains with deletions covering the bacterial *tonB* gene and extending for varying distances into the prophage (FRANKLIN *et al.* 1965). One class of deletion mutants gave reduced phage yield after induction, but no loss of any essential phage function, as judged either by gene rescue from the prophage or by the competence of the progeny plaque formers.

This type of deletion, having only a quantitative effect upon the yield of induced prophage, was supposed to have impaired the mechanism for prophage excision, by removing one of the two junctures of the prophage genome with the bacterial genome, i.e., the hypothesized (CAMPBELL 1962) homology regions. We

therefore call this a deletion of a prophage terminal, and further substantiate this interpretation with results presented here.

When a lysogen for a prophage with a terminal deletion is induced by ultraviolet (UV) irradiation, all phage functions essential for maturation appear to proceed normally (DOVE 1966; FRANKLIN, unpublished); the cells lyse, yielding inactive phage particles in large numbers (GRATIA 1966) as well as plaque-forming particles in numbers 100 to 1,000 $\times$ fewer than normal (FRANKLIN *et al.* 1965). It is these exceptional plaque-formers which are the material of the present study.

When centrifuged in a CsCl density gradient, the plaque-formers from a terminal deletion prophage show a wide range of buoyant densities. Unlike the parental phage which forms a sharp band at a position slightly less dense than λ (Figure 2), plaque-formers from terminal deletion prophages are distributed in density from 1.507, the parent phage density, to 1.474; the upper and lower limits of the density distribution are sharply defined (Figure 3). Although there are, characteristically, four peaks along the density distribution of plaque-formers, a continuous spectrum of phages with reduced density can be isolated.

Plaques obtained from any position along the density gradient give rise to a series of phage clones of characteristic, heritable density (Figure 4). In general, each clone is at least 90% pure with respect to density, but a certain degree of instability in density is observed and will be discussed below. The genetic stability of these low-density plaque-forming phages stands in contrast to the high frequency of restoration reported by FRASER (1966) for low-density λ phages arising from a different situation.

The DNA content of low-density mutants: The alterations in phage density can be shown to be attributable to the DNA content of the phage particles. Small differences in the molecular weight of DNA of each phage clone can be neatly assessed by the method of BALDWIN *et al.* (1966). This method depends upon the cohesive ends of the DNA of vegetative lambdoid phages (HERSHEY and BURGI 1965; KAISER and INMAN 1965). These cohesive ends allow a specific interaction between the ends of different lambdoid DNA molecules, giving rise to dimers, trimers, etc. For the purpose of measuring the size of an unknown molecule of DNA from λ or a lambda-related phage, λ DNA labeled with 5-bromouracil (BU) is allowed to dimerize with the unlabeled DNA of unknown molecular weight. The buoyant density of the hybrid molecules, measured in CsCl gradient, then bears a linear relationship to the size of the unlabeled DNA, which has been added to a constant length of BU-DNA from standard λ phages (BALDWIN *et al.* 1966).

For $i^{\phi 80}h^{+\lambda}$ and each of four low-density mutants studied, the bands of DNA in cesium chloride density gradients are sharply defined (UV optics, ultracentrifuge), indicating a uniformity of molecular size for each. That the DNA from low-density phages retains both cohesive ends is evidenced in the existence of DNA bands of hybrid density equivalent to dimers and *both* trimers (where one trimer is BU-λ : $i^{\phi 80}h^{+\lambda}$: BU-λ, the other is $i^{\phi 80}h^{+\lambda}$: BU-λ : $i^{\phi 80}h^{+\lambda}$). The positions of these bands show that the phages of each low-density clone contain less DNA
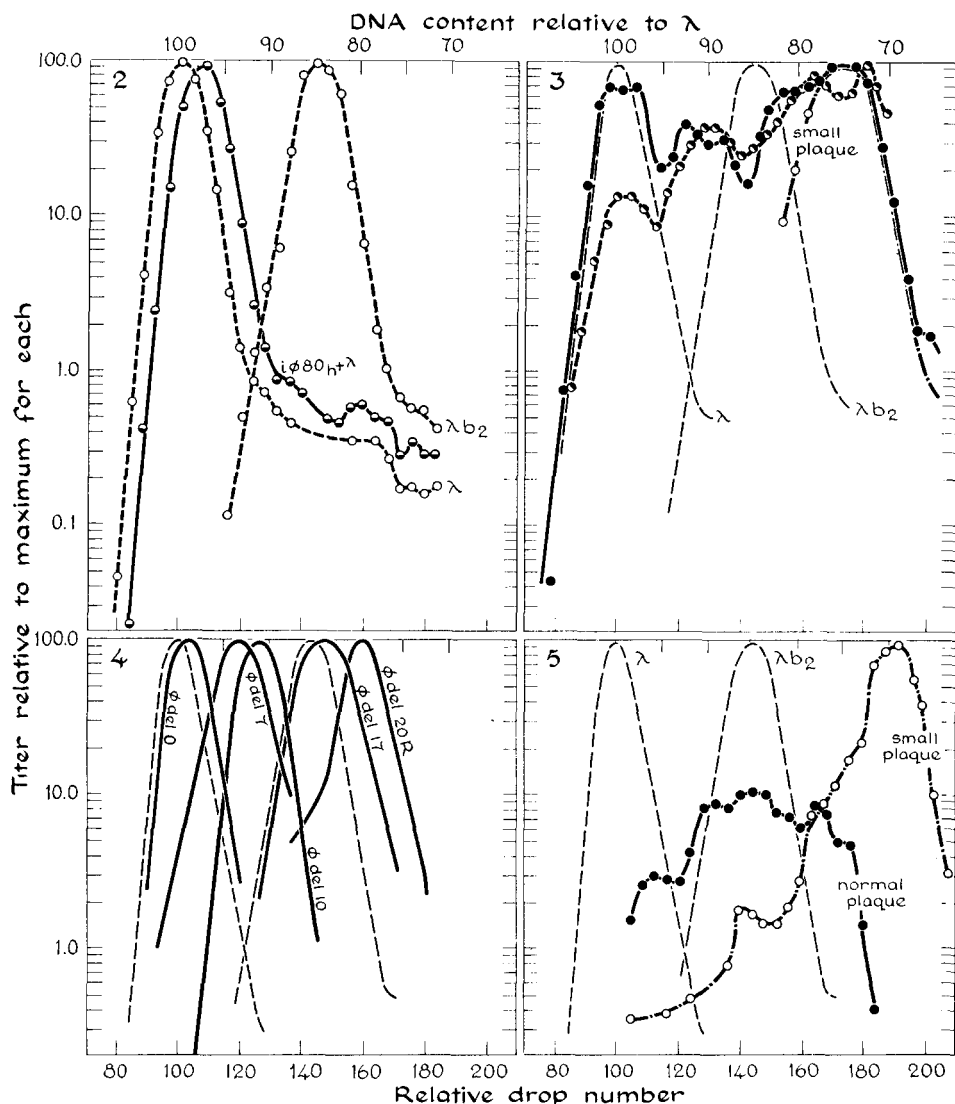
FIGURES 2–5.—The distribution of plaque-forming phages in cesium chloride density gradients. Standard phages λ and λb2 were added to each gradient.   FIGURE 2.—The density distribution of $i\phi80h+^\lambda$ induced by UV from a normal lysogen.   FIGURE 3.—The density distribution of $i\phi80h+^\lambda$ induced by UV from a terminal deletion lysogen. Two independent lysates are shown (● and ○). At the lowest densities the plaques formed are all smaller than normal.   FIGURE 4.—A composite of several independent gradient centrifugations, each of a different phage lysate derived from single plaques picked from various positions along the distribution curves of Figure 3.   FIGURE 5.—The density distribution of plaque formers in a plate lysate started with a single small plaque from ϕtdel 33.

than the parental $i\phi80h+^\lambda$ phage. The amount of the DNA loss in each case is sufficient in itself to account for the reduced densities of the phage particles (WEIGLE, MESELSON and PAIGEN 1959) (Table 1). Studies of other low-density

TABLE 1

Phage density accounted for by DNA content

Phage	Fractional loss of DNA relative to $\lambda = a^*$	Density change (g/cm³) calculated from $\alpha\dagger$	Density change relative to λ measured in density gradients
$i^{\phi 80}h^{+\lambda}$	0	0	—.001
ϕtdel 10	.08	—.009	—.010
ϕtdel 20R	.17	—.020	—.020
ϕtdel 23	.22	—.026	—.023
ϕtdel 33	.28	—.033	—.034
λb2c	.12	—.013	(—.017 = standard)

* Measured by method of BALDWIN *et al.* (1966).
† KELLENBERGER, ZICHICHI and WEIGLE (1961).

λ mutants (KELLENBERGER, ZICHICHI and WEIGLE 1961) and of λdg phage particles (WEIGLE, MESELSON and PAIGEN 1959; KAYAJANIAN and CAMPBELL 1966) have also shown a correlation between particle density and DNA content. We shall therefore henceforth consider that the density of every phage derived here from $i^{\phi 80}h^{+\lambda}$ is directly related to the size of its DNA molecule. The DNA reduction has been calculated from the buoyant density in terms of percent loss relative to λ (since $i^{\phi 80}h^{+\lambda}$ is itself very close to λ in density), and each phage type is named accordingly: ϕtdel 0, ϕtdel 1, ϕtdel 5, 7, 10, 11, 17, 20R, 22, 23, 33. So far no two isolates have had the same density, but no effort has been made to exhaust the possible types.

Genetic measure of the size and linkage of deletions in $i^{\phi 80}h^{+\lambda}$: The deletion of DNA from low-density phage, and the site of that deletion, has been confirmed by genetic measurements of the linkage of h to c in $i^{\phi 80}h^{+\lambda}$, and in several of the deletion phages derived from it. In order to effect these linkage studies, spontaneous mutant forms of $i^{\phi 80}h^{+\lambda}$ were isolated successively: (a) a clear mutant, $i^{\phi 80}c\,h^{+\lambda}$; (b) an extended host range mutant, $i^{\phi 80}c\,h^{\lambda}$, able to infect certain λ-resistant bacteria. The $i^{\phi 80}c\,h^{+\lambda}$ mutant was itself found to have reduced density, equivalent to a 3% loss of DNA relative to $i^{\phi 80}h^{+\lambda}$. This density loss proved inseparable from the *clear* phenotype. [Apparently it does not affect any essential genes, such as N which in λ is linked to c, lying between $cIII$ and cI (EISEN *et al.* 1966).]

The degree of linkage of c^+ to h^+ in $i^{\phi 80}h^{+\lambda}$ and its terminal deletion phages was studied in crosses to $i^{\phi 80}c\,h^{\lambda}$ ($= h\,c$). The frequency of $h\,c^+$ recombinant progeny relative to $h\,c$ parent-type progeny was surprisingly high (30%) (see KAISER 1955; JORDAN 1964) in crosses to $i^{\phi 80}h^{+\lambda}$. It was significantly reduced (to as low as 1 to 3%) in parallel crosses to terminal deletion phages (Table 2). This measurement of genetic shrinkage between h and c in $del \times del^+$ crosses should approximate the same assessment in $del \times del$ crosses, although we cannot estimate the effect of the del-del^+ inhomogeneity here upon recombination in adjacent regions.

The progeny of crosses between $i^{\phi 80}c\,h^{\lambda}$ and $i^{\phi 80}h^{+\lambda}$ or its terminal deletion

TABLE 2

Recombination frequencies between h and c, comparing iϕ^{80}h$+\lambda$ (h$+$c$+$) *to deletion phages derived from it, in crosses to* iϕ^{80}chλ (hc)

Parent h$+$c$+$	Expt. No.	Percent recombination hc$+$/(hc$+$ + hc) ×100	Proportion of hc$+$ at		Distances as % of total DNA‡			
			Low density* rec. h-del	High density† rec. del-c	h-del	del	del-c	Sum
iϕ^{80}h$+\lambda$	1	21	..	..	..	..	..	..
	2	31¶	0	100	..	0	..	31
	2	27	..	..	..	..	..	..
ϕtdel 7	1	23	..	..	..	7	..	30
ϕtdel 10	1	31	..	..	..	..	..	..
	1	28¶	7	93	2	10	26	38
	2	30	..	..	..	..	..	..
ϕtdel 17	1	7.4	..	..	..	..	..	..
	1	9.1¶	55	45	5	16	4	25
ϕtdel 23	1	3.4	..	..	..	..	..	..
	1	5.9¶	30	70	2	21	4	27
	2	6.6¶	31	69	2	21	5	28
	3	9.6	..	..	..	..	..	..
ϕtdel 22	1	6.5¶	69	31	4.5	20	2	27
ϕtdel 33§	1	1.2	..	..	..	..	..	..
	1	2.4¶	..	..	..	..	..	..
	2	3.6¶	25‖	75	1	28	3	32
	3	2	..	..	..	..	..	..
ϕtdel 20R	1	2.0	..	..	..	..	..	..
	1	1.2¶	80	20	1	17	0.2	18
	2	3.7¶	88	12	3	17	0.4	20
	3	1.5	..	..	..	..	..	..
ϕtdel 1	1	5.7	..	..	..	1	..	7
	1	3.2¶	..	..	..	1	..	4

The cross procedure is as given for Table 4, except that W1485 was used as host. Progeny phages were adsorbed to W1485 at a multiplicity ≪1 in order to unmix phenotypes. These infected bacteria were then diluted and plated at 34°C on W1485 to count clear and centered plaques, which are primarily parental and present in near equal numbers, or on CR63 to count clear hc parental and centered hc$+$ recombinant phages. The ratio of recombinant to total phages on CR63 was used to calculate the recombination frequency. Infection by either parent alone gave no hc$+$ progeny, i.e., less than 0.1% of the lysate titer. Lysates from mixed infections were centrifuged in CsCl density gradients with λ and λ b2 marker phages, and the distributed phages titered as above by pre-adsorption to W1485 and plating on CR63 at 34°C.

* Recombinants with the low density of the parental deletion phage are formed by an exchange between h and deletion as at (1) in the diagram below.

† Recombinants with the high density of iϕ^{80}h$+\lambda$ are formed by an exchange between deletion and c as at (2) in the diagram.

susA	h$+$	del	c$+$	susR
	/(1)	(2)/		
h	del$+$	del c		

‡ The length of the DNA deletion is calculated from the phage density (Table 1; KELLENBERGER, ZICHICHI and WEIGLE 1961). The length of DNA measured by recombination frequency is roughly estimated on the following basis: the h to c interval includes at least 30% of the phage's DNA, since that is the longest deletion found; maximum recombination between h and c is about 30%; therefore let 1% recombination represent 1% of phage's DNA, as an *average* estimate.

§ The stock of phage ϕtdel 33 used in crosses contained 10% of higher density revertant phages, of which ϕtdel 20R is one (see text).

‖ Low density recombinants from ϕtdel 33 have the small plaques characteristic of ϕtdel 33.

¶ These recombination frequencies are calculated from the peak titers after CsCl density gradient centrifugation. The other figures come from direct titration of cross lysates, using duplicate plates.

phages could be further characterized with respect to their density. Each lysate from mixed infection was centrifuged to equilibrium in CsCl, and the numbers of $h\,c^+$ and $h\,c$ phages titered through the gradient. Here density serves as a third genetic marker, allowing measure of the recombination frequency from h to deletion, and from deletion to c (JORDON 1964). In each cross $h\,c^+$ recombinant progeny were found both at wild-type density and at the density of the h^+c^+ parent, showing that none of the terminal deletions overlap the 3% DNA loss from $i^{\phi80}c\,h^{+\lambda}$. The proportion of $h\,c^+$ high density to $h\,c^+$ low density gives an estimate of the position of the deletion with respect to h and c (Table 2). This determination, being internally controlled, should be independent of many external variables affecting phage crosses.

The results of recombination measurements between h and c in normal and deletion phages can be used to make a genetic map of the deletions with respect to the phage genome. It is assumed that during vegetative infection recombination between h and c occurs in the center region of the vegetative lineom (see diagram, Table 2). By converting both recombination frequencies and phage densities to approximate lengths of DNA, a scale map of the central region of $i^{\phi80}h^{+\lambda}$ and its deletion mutants can be drawn (Figure 6). In assigning DNA lengths to represent recombination frequencies (Table 2), the assumption is made that recombi-
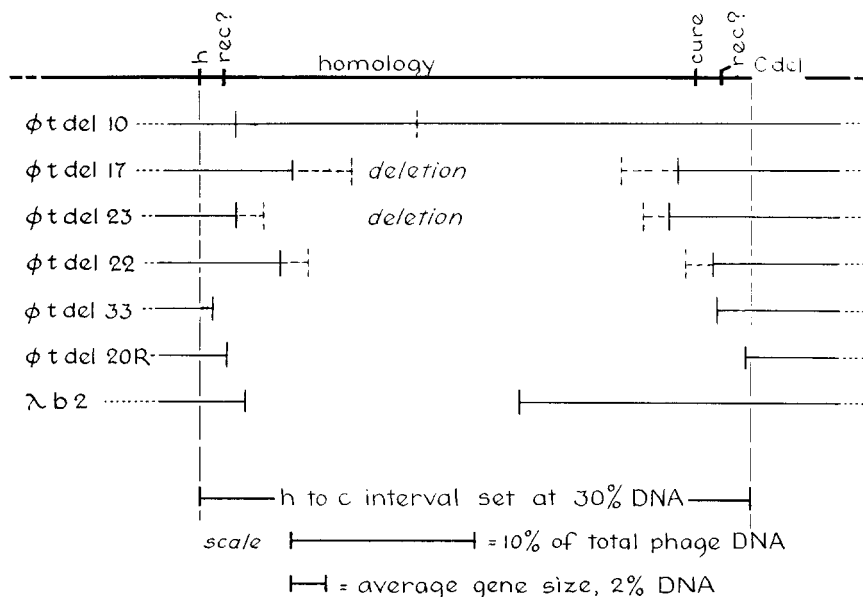

FIGURE 6.—Genetic map of the central region, drawn to scale. Shown are the extent of deletions in several low-density phages derived from a terminal deletion prophage of $i^{\phi80}h^{+\lambda}$. Deletion limits are set on the basis of recombination frequencies to h or to c (———) (Table 2). Resulting discrepancies from physical measure of deletion size (Table 1) are indicated (– – – – –). φtdel 1 is not shown because its characteristics are aberrant and its near-normal density measurement of its deletion's recombination to h and c. The mapping of λb2 is based upon JORDAN (1964). The placement of *cure* and *recombination* genes is based upon the presence or absence of these functions in the various deletion phages.

nation occurs randomly throughout the center region of the phage genome. A bias may be indicated, however, by the relatively high total DNA distances calculated for $i^{\phi80}h^{+\lambda}$, ϕtdel 7 and ϕtdel 10, reflecting relatively high recombination between h and c. It has been proposed by E. SIGNER (personal communication) that these phages contain a region of accentuated recombination which is missing in phages with larger deletions. If there is a region of high recombination, then the DNA distances from deletions to h or c are underestimated. The fact that ϕtdel 10, despite its deletion, shows even greater recombination between h and c than does $i^{\phi80}h^{+\lambda}$ is enigmatic. Other exceptional phages, ϕtdel 1 and ϕtdel 20R (the latter derived from ϕtdel 33), show relatively low total DNA distances between h and c; these will be considered below. In the other four cases the reduction of DNA in low-density phages is expressed proportionally as reduced genetic distance between h and c markers. There is apparently considerable latitude in the points at which the DNA can be cut at both sides of the center region of the vegetative phage genome.

Instability in phage density: Although the lysates of the deletion phages are usually well over 90% uniform with respect to density, the initiation of new lysates from single plaques has occasionally led to shifts in the density property. The situation seems most approachable in the case of ϕtdel 33, by far the most unstable in the density property. ϕtdel 33 has the largest deletion yet isolated and is distinguished from all the other deletion phages by its small plaque size. Each lysate derived from a single small plaque of ϕtdel 33 contains from 1 to 50% of normal-sized plaques which prove to have greater density that that of ϕtdel 33 (Figure 5). These revertants occur even if the host bacterium has a deletion covering the ϕ80 attachment site. In the case of revertant ϕtdel 20R, the new density was shown to correspond to a new DNA molecular weight (Table 1). Yet the added DNA probably does not restore any part of the deleted DNA: (a) because phages equivalent to ϕtdel 33 recur spontaneously in lysates of ϕtdel 20R; (b) the c-h recombination frequency is as low in ϕtdel 20R as it is in ϕtdel 33; (c) none of the functions missing from ϕtdel 33 (see below) is restored in the higher-density revertants. The restored DNA very likely lies in the region to the right of the deletion, since recombinants between h and del all have the density of ϕtdel 20R, none of ϕtdel 33. Furthermore the frequency of high density $h\,c^+$ recombinants from ϕtdel 20R (crossover between del and c) is reduced about tenfold relative to ϕtdel 33 (Table 2), leading to the suggestion (E. SIGNER, personal communication) that the majority of this recombinant class is nonviable owing to a DNA length too great to be packaged, the sum of the normal $h - c$ length plus the 13% added DNA in ϕtdel 20R. If the added DNA were to the right of c, then it could be removed by a second recombination event, giving the observed small class of $h\,c^+$ recombinants at the density of $i^{\phi80}h^{+\lambda}$. Somehow, DNA can be added to the phage genome during vegetative replication. The source of this DNA, its location in the genome, and the mechanism of its addition are of interest.

Functions lost in deletion phages: We have seen that as much as 30% of the DNA complement of phage $i^{\phi80}h^{+\lambda}$ can be deleted from the center of the vegeta-

tive genome without loss of the ability of the phage to form plaques. Lysates of these phages all give normal high titers, and the plaque morphology is normal in all cases except ϕtdel 33 which forms a small plaque. Single-step growth curves of ϕtdel 23 and ϕtdel 20R in either AB1157 Rec$^+$ or AB2463 Rec$^-$ showed normal latent period, growth rate and burst size, compared to standard $i^{\phi 80}h^{+\lambda}$. ϕtdel 33 has a normal latent period, but somewhat reduced burst size. Thus at least 30% of the phage genome plays no detectable role in vegetative growth and maturation.

On the other hand, comparative studies of the deletion and standard phages have exposed several functions which are dependent upon part of this central region of the genome. In the first place, of the plaque forming units derived from a terminal deletion prophage none is able to establish stable lysogeny. As with the deletion phage λb2 (KELLENBERGER, ZICHICHI and WEIGLE 1961), plaques are turbid, indicating the functioning of the temperate phage immunity function. Yet repeated restreaking of phage-yielding colonies do *not* yield stably lysogenic clones.

The ability to cure by superinfection (JACOB and WOLLMAN 1957; SIGNER and BECKWITH 1966) was tested for $i^{\phi 80}h^{+\lambda}$ and each of its deletion mutants. For this purpose a lysogen for another ϕ80-λ recombinant was used, $i^{\lambda}cI857h^{+\phi 80}$, which also localizes on the *E. coli* chromosome close to *tryp* and *tonB*, but has the immunity of λ and is heat-inducible (SUSSMAN and JACOB 1962). The elimination of prophage $i^{\lambda}cI857h^{+\phi 80}$ from a lysogen can be readily assessed by the gained resistance of the bacterium to elevated temperatures: lysogens for $i^{\lambda}cI857h^{+\phi 80}$ are induced at 43°C, resulting in cell lysis; non-lysogens replicate at 43°C, forming colonies. Of nine deletion phages tested, five are like $i^{\phi 80}h^{+\lambda}$ in their ability to cure $i^{\lambda}cI857h^{+\phi 80}$ (Table 3). The four deletion phages which have lost this ability include ϕtdel 22 and ϕtdel 33 with the longest deletions, as well as ϕtdel 20R and ϕtdel 1 which show genetic evidence of long deletions (Table 2) despite their higher DNA content. Neither $i^{\phi 80}h^{+\lambda}$ nor any of its deletion phages can cure $\lambda cI857$ from its chromosomal location near *gal*, confirming the specificity to attachment site which has been reported for this superinfection-curing phenomenon (SIGNER and BECKWITH 1966).

Also examined was the ability of $i^{\phi 80}h^{+\lambda}$ and its deletion phages to recombine in a Rec$^-$ bacterium (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966). For this purpose it was necessary to isolate mutants from each of ϕtdel 23 and ϕtdel 33, in order that self-crosses could be made for each type. The genetic region to be screened for recombination was chosen so as to be independent of the region carrying the deletions. In addition, genetic markers were chosen such that recombinants could be selectively assayed. In crosses of *susAB h* $\times$ *susA$^+$B$^+$h$^+$*, only recombinants *susA$^+$B$^+$h* can plate on a non-permissive, h^+-resistant bacterium, and the recombination event is within the left arm (*susA-h*) of the genome.

Extended host-range mutants (*h*) were selected on *E. coli* CR63. Their frequency ranged from 10^{-5} to 10^{-9} in lysates of different deletion phages, 10^{-8} in $i^{\phi 80}h^{+\lambda}$. From among a considerable variety of plaque morphologies, those were

TABLE 3

Cure of prophage by superinfection with heteroimmune phage, entire or deletion

Superinfecting phage	W1485 ($\lambda c1857$) Colonies/ml at 43°C/ Colonies/ml at 34°C		Cure	W1485 ($i\lambda c1857h\phi^{80}$) Colonies/ml at 43°C/ Colonies/ml at 34°C		Cure
$i\phi^{80}h^{+\lambda}$	9×10^{-6}		—	9.1×10^{-1}	. . . .	+
ϕtdel 0	$<2 \times 10^{-6}$		—	1.3×10^{-2}	7.8×10^{-3}	±
ϕtdel 7	3.3×10^{-5}		—	4.3×10^{-2}	5.6×10^{-2}	±
ϕtdel 10	$<5 \times 10^{-6}$		—	2.1×10^{-1}	5.1×10^{-1}	+
ϕtdel 17	$<3 \times 10^{-6}$		—	4.8×10^{-1}	1.1	+
ϕtdel 23	. . . .		. .	9.3×10^{-2}	. . . .	+
ϕtdel 23 h $susAB$	1.7×10^{-5}		—	2.0×10^{-1}	. . . .	+
ϕtdel 22	7.4×10^{-6}		—	1.8×10^{-5}	2.7×10^{-3}	—
ϕtdel 33	. . . .		. .	. . . .	5×10^{-3}	—
ϕtdel 33 h $susAB$	1×10^{-5}		—	5.6×10^{-5}	. . . .	—
ϕtdel 20R	6.3×10^{-5}		—	4.8×10^{-5}	. . . .	—
ϕtdel 1	. . . .		. .	. . . .	4.2×10^{-3}	—
none	1×10^{-5}		—	5.2×10^{-5}	2.9×10^{-4}	—

The lysogens were grown at 25°C in broth to about 2×10^8/ml, washed in 10^{-3} M Mg^{++}, superinfected (or not) at a multiplicity of about 50, diluted 400× into broth and shaken at 25°C for 4 hours. Survivors were then plated at 34°C or at 43°C. Bacteria which retain the temperature-inducible prophage are unable to form colonies at 43°C; cured bacteria grow equally well at 43° or 34°C.

selected whose lysates contained the highest titers. A striking peculiarity of the h mutants selected particularly from deletion phages is their instability: a high proportion (up to 30%) of h^+ revertants occurs spontaneously, so that h lysates are always prepared on CR63. This reversion to h^+ does not, however, affect the assessment of h recombinants.

Into the h mutants was crossed the $susAB$ double marker from $i^{\phi 80}h^{+\lambda}susAB$, the latter having been obtained from a cross of $\lambda c1857h$ $susAB \times i^{\phi 80}h^{+\lambda}$, where the left arm of $i^{\phi 80}h^{+\lambda}$ is derived from λ. In these crosses turbid plaques of h^+ were distinguished from clear plaques of h on a mixed indicator of 5 parts of W1485: 1 of CR63. (The c^+ character does not contribute significantly to plaque turbidity under these circumstances.) Phages with $susAB$ were detected by their inability to plate on non-permissive W3101, their ability to plate on permissive W1485. The immunity type is identified by test against λ or $\phi 80$ lysogens. In each case the $susAB$ h derivative maintained the deletion of the initial phage, as shown by inability to lysogenize, capacity or incapacity to cure by superinfection (Table 3), as well as by the recombination characteristics given below.

Initial self-crosses of ϕtdel 23 $\times$ ϕtdel 23 $susAB$ h and of ϕtdel 33 $\times$ ϕtdel 33 $susAB$ h showed that $susA^+B^+h$ recombinants could be selected from ϕtdel 23 infecting either a Rec$^+$ or a Rec$^-$ bacterium, but from ϕtdel 33 only when it infected a Rec$^+$ host. Controls showed that the parental phages were both present in the lysates of both Rec$^+$ and Rec$^-$ bacteria, and that infection by either parent alone gave 10^{-3} or less of recombinant types, when compared to a recombining mixed infection. Indications were, therefore, that ϕtdel 33 is missing a recombination function which is present in ϕtdel 23, which is substituted by the rec^+ allele

of *recA13* in AB2463, and which is nonessential to phage replication even in Rec⁻AB2463.

Further tests of phage recombination function were made in comparative crosses of all of the original deletion phages to ϕtdel 23 *susAB h* and to ϕtdel 33 *susAB h* in Rec⁺ or in Rec⁻ bacteria (Table 4). Only ϕtdel 33, its "revertant" ϕtdel 20R, and ϕtdel 1 gave no recombinants when crossed in Rec⁻ to ϕtdel 33 *susAB h*, although their crosses to ϕtdel 23 *susAB h* did yield recombinants. We would say, therefore, that the DNA deleted from ϕtdel 33 and ϕtdel 1 includes a gene or genes determining a phage recombination function. This function can

TABLE 4

Recombination between susAB *and* h *markers of deletion phages crossed in* Rec⁺ *or* Rec⁻ *bacteria*

Parental phages		Rec⁺ bacteria			Rec⁻ bacteria		
h⁺ susA⁺B⁺	*h susAB*	*rec⁺* gene dose	Percent recombination		*rec⁺* gene dose	Percent recombination	
$i\phi^{80}h+\lambda$	ϕtdel 23	3	1.8		2	4.8	
$i\phi^{80}h+\lambda$	ϕtdel 33	2	3.6		1	2.5	
ϕtdel 0	ϕtdel 23	3	2.8		2	3.8	
ϕtdel 0	ϕtdel 33	2	4.2		1	2.1	
ϕtdel 10	ϕtdel 23	3	0.9		2	3.7	
ϕtdel 10	ϕtdel 33	2	4.1		1	3.3	
ϕtdel 17	ϕtdel 23	3	2.4		2	4.1	
ϕtdel 17	ϕtdel 33	2	4.3		1	4.1	
ϕtdel 22	ϕtdel 23	3	2.1		2	3.4	
ϕtdel 22	ϕtdel 33	2	4.6		1	2.0	
ϕtdel 23	ϕtdel 23	3	2.6	2.3	2	2.7	2.1
ϕtdel 23	ϕtdel 33	2	0.6	1.2	1	1.2	1.1
ϕtdel 33	ϕtdel 23	2	2.6	3.9	1	1.4	2.6
ϕtdel 33	ϕtdel 33	1	1.8	4.2	0	<0.01	<0.04
ϕtdel 20R	ϕtdel 23	2	5.1		1	2.9	
ϕtdel 20R	ϕtdel 33	1	2.2		0	<0.07	
ϕtdel 1	ϕtdel 23	2	3.8	3.6	1	4.7	3.8
ϕtdel 1	ϕtdel 33	1	.3.6	5.9	0	<0.01	<0.03

Parental phages at a multiplicity of about 5 for each were adsorbed to AB1157 Rec⁺ or AB2463 Rec⁻ bacteria (1 to 3×10^8/ml) at 37°C for 20 minutes; adsorption for each parent was at least 90%. Unadsorbed phage were inactivated during 10 minutes at 37°C by anti-λ serum diluted to K=0.5. Infected bacteria were then titered, diluted 200× into warm broth and aerated for 2 hours at 37°C. Lysates were shaken with chloroform and centrifuged at low speed.

Progeny phages were titered by adsorbing at a multiplicity of ≪1 to W1485 or to W3101 in order to unmix phenotypes. These singly infected bacteria were then diluted and plated on W3101/λ to select *h sus A⁺B⁺* recombinants, or on a 5:1 mixture of W1485 and CR63 to distinguish parental types. Recombination frequency is calculated from $2\times h$ *sus A⁺B⁺*, as percent of the total number of phages in each lysate.

The titers of progeny phages from Rec⁻ bacteria tend to be lower than from Rec⁺ bacteria. This is apparently due to the fact that cultures of Rec⁻ contain 20 to 70% nonviable cells which are incapable of reproducing phage. If phage yields are calculated per phage-yielding bacterium (burst size), the yields from Rec⁺ and Rec⁻ are comparable; burst sizes of *h⁺ sus A⁺B⁺* were 50 to 200 in Rec⁺, 24 to 204 in Rec⁻; burst sizes of *h sus A B* were 64 to 116 in Rec⁺, 44 to 140 in Rec⁻. Furthermore, burst sizes of Rec⁻ phages in Rec⁻ host (24 to 78 for *h⁺ sus A⁺B⁺*, 50 to 104 for *h sus A B*) approximated those for Rec⁺ phages in Rec⁻ host (66 to 204 for *h⁺ sus AB⁺*, 44 to 140 for *h sus AB*).

Because the Rec⁺ and Rec⁻ hosts are non-permissive, the *h sus* parental phage will only reproduce in cells which have been co-infected with *sus⁺* phage. Thus the near equal frequency observed for *h sus* and *h⁺sus⁺* progeny in lysates is evidence for good multiple infection.

be supplied by a less-deleted co-infecting phage, or by the host bacterium. In crosses where the recombination function was represented by 1, 2 or 3 gene doses, in the bacterium or either phage parent, or any combination of these, no quantitative effect on the recombination frequency between *susAB* and *h* was observed (Table 4). Thus a single Rec$^+$ representative, in either phage or bacterium, supplies a function which permits the recombination of vegetatively replicating phage genomes, with a frequency not limited by the Rec function.

The inability of ϕtdel 33 and the ability of ϕtdel 23 to recombine in Rec$^-$ AB2463 proved to hold true also in bacteria with independent Rec$^-$ mutations: Rec$^-$AB2462, AB2470 (partial effect), JC4451. If these bacterial Rec$^-$ mutations actually represent independent functions involved with recombination, then all of these functions must somehow be covered by the phage's own capacity for recombination.

A few unsuccessful attempts have been made to allow the phage recombination function(s) to substitute for bacterial functions lost in Rec$^-$ mutants.

(1) No alleviation of the severe UV-sensitivity of AB2463 (CLARK and MAR-GULIES 1965; HOWARD-FLANDERS and THERIOT 1966) was achieved by infecting irradiated AB2463 with normal or UV-killed ϕtdel 23 at multiplicity of 1.

(2) The survival of $i^{\phi 80}h^{+\lambda}$, ϕtdel 23 and ϕtdel 33 after UV irradiation is the same, even if the survivors are assayed on Rec$^-$ bacteria. There is thus no indication that the center region of the phage genome can play a role in UV-survival mechanisms.

(3) The low efficiency of generalized transduction of Rec$^-$ bacteria by phage P1 (HERTMAN and LURIA 1967) could not be increased by co-infecting with ϕtdel 23 grown on a P1 lysogen. The inability of phage Rec$^+$ to function in this way would be consistent with the picture of phage Rec$^+$ drawn by GOTTESMAN and YARMOLINSKY (1967).

DISCUSSION

The occurrence of plaque-forming phages with chromosomal delections in the center can be rationalized on the basis of CAMPBELL's model of lysogeny (1962). In this model, each prophage terminal is part of the homology region common to phage and bacterium, by means of which the phage genome is integrated into the continuity of the bacterial chromosome by a single recombination event at lysogenization, or released upon induction of the prophage. If the normal release mechanism depends upon interaction between the two prophage terminals, then the loss of one terminal, by chance deletion from the prophage state, would leave the possibility for aberrant release events only. Phage genomes might then be cut out from within the residual prophage genome, or they might include the remaining prophage terminal as well as adjacent regions of the bacterial genome. Our selection for plaque-forming particles would eliminate all products lacking in functions essential for phage reproduction and maturation. We recover rare infective phages. Low frequency release has also been observed from single lysogens of λb2 (FISCHER-FANTUZZI, 1967) and of λ*integration* (GOTTESMAN

and YARMOLINSKY 1967). The few infective phages recovered from a terminal deletion prophage contain DNA in amounts 70 to 100% of that in the normal phage particle. None of the rare infective phages is able to lysogenize. On the one hand this is consistent with the deletion of one terminal from the progenitor prophage. On the other hand it says that either the second terminal is eliminated during abnormal excision, or that in these terminal deletion phages the inclusion of the second terminal does not suffice for lysogenization. The latter could result if the second terminal is structurally inadequate or if some other phage function needed for lysogenization has been lost. Particles including adjacent bacterial DNA can be formed, since lysates of a terminal deletion prophage do transduce the *tryp* genes, at a frequency 10^{-3} times the frequency of plaque-formers in these lysates. [The total number of *tryp*-transducers in these lysates is the same as in a lysate from a normal lysogen.] The lower frequency of the transducing particles suggests that their formation is more stringent than that of the non-lysogenizing infective phages, though both are examples of abnormal excision. Possible initial events following induction may include scission at the second terminal, replication of phage DNA and covalent bonding of the prophage subterminal regions, in unknown sequence.

The deletions in our mutant plaque-forming phages lie at the center of the vegetative genetic map, as shown in several cases by measurements of recombination between h and c in vegetative crosses. It is this region which lies terminally in the circularly permuted prophage genome (CALEF and LICCIARDELLO 1960; FRANKLIN et al. 1965), divided at the homology region by the insertion event according to the CAMPBELL model (Figure 1). Genetic measurements here, of the linkage of the central deletions to c and h, show that one end lies as much as 5% total DNA from h, as in ϕtdel 17 and ϕtdel 22 the other end as much as 4% total DNA from c, as in ϕtdel 17 and ϕtdel 23 (Figure 6). The ability to lysogenize is lost from all deletion phages, so that the region of homology must extend within the length common to all deletions, between 5% from h and 4% from c, the general location already designated by the position of the b2 mutant of λ (JORDAN 1964), as well as of the genetic determinant of chromosomal location in phages λ and 21 (LIEDKE-KULKE and KAISER 1967). The limits of the homology region can not yet be defined.

The differences in the linkage of independent deletions to h or to c does show that in many cases the deletion plaque-formers are formed variously from within the prophage, and do not include the second terminal. A possible exception is ϕtdel 10, which has lost 10% of its DNA and yet shows even greater recombination between h and c than does normal $i^{\phi 80}h^{+\lambda}$ (Table 2). Conceivably ϕtdel 10 was so excised as to include the intact homology region which lies distal to the deletion in the terminal deletion prophage (R. FREEDMAN, personal communication). The inclusion of this intact homology region near to c might account for the excess h-c recombination, all of which seems to occur between del and c, rather than between h and del. Apparent contradiction to this proposal lies in the inability of ϕtdel 10 to lysogenize, despite the fact that it lacks neither of the other center functions measured here.

The amount of DNA in the particles of each mutant phage clone has uncertain significance. It does not exceed that of the parental $i^{\phi 80}h^{+\lambda}$, nor does it fall below 70% of the DNA content of $i^{\phi 80}h^{+\lambda}$ (Figure 3). The upper limit probably does not simply represent a maximum phage head content, since λdg's (which depend upon the same coat determinants as does $i^{\phi 80}h^{+\lambda}$) are known which exceed the present maximum by 5 to 8% (KAYAJANIAN and CAMPBELL 1966; WEIGLE, MESELSON and PAIGEN 1959). The lower limit is well below any previously reported, and could represent either a minimal amount of DNA for stable packaging or a minimal amount of DNA to provide all essential functions. Between these two limits there is a continuous distribution of DNA contents, though four levels seem to be especially favored (Figure 3). Although each clone shows genetic constancy in its DNA content, there is evidence that secondary alterations may occur, so that in any particular case it is not certain whether the DNA content observed is the result of the primary excision event or some subsequent modification. Thus ϕtdel 1, which apparently has a full DNA complement, behaves genetically and physiologically as though a large segment is missing.

In addition to the region of homology, we have tried to identify other functions of the center genome. The ability to cure another prophage with the same attachment site specificity was lost in ϕtdel 22 as well as in ϕtdel 33. A function promoting the genetic recombination of phage genomes was lost in ϕtdel 33 but not in ϕtdel 22. Phages with deletions apparently less extensive than these retained both functions. On the basis of genetic linkage of deletions 22, 23 and 33 to c and h, we would locate the curing function near to c and the recombination function close to h (Figure 6). But it is also possible that the recombination function lies between *cure* and c, or that it has two components, one linked to c and the other to h. It should be possible to distinguish these possibilities by studying transducing phage particles, where in one type (λd*bio*, ϕ80d*tryp*) the entire segment from homology to c would be deleted, while in the other (λd*gal*, ϕ80d*su*) the segment from homology to h would be deleted (see SIGNER and BECKWITH 1966). Clearly, a large portion of the center genome is still uncharted.

Comparison may be made of the center map developed here with other reports of center-located lambda functions. The mutations of a few defective lysogens have mapped between h and c (JACOB, FUERST and WOLLMAN 1957; FUERST and MOUNT 1966). Of these, we have examined λt50 and found that of the few plaque forming units released upon induction, none is able to lysogenize. This suggests that t50 is a lesion of a lysogenizing function, or of the phage homology region, similar to the prophage terminal deletion described here. Point mutants of λ, called *integration* because they are unable to lysogenize, have recently been reported, mapping between h and c, linked to c (GOTTESMAN and YARMOLINSKY 1967; ZISSLER 1967). In addition, comparison of different λ-ϕ80 hybrids has permitted the mapping of phage exonuclease and β-protein between t50 and c (RADDING, SZPIRER and THOMAS 1967). A small plaque s mutant was mapped near to h by KAISER (1955). In accord with results reported here, none of these functions is essential for vegetative growth.

Evidently the curing and integration functions are two facets of the attach-

ment-site-specific function proposed by SIGNER and BECKWITH (1966). The integration character of λ shows attachment site specificity, and integration mutants are unable to cure by superinfection (GOTTESMAN and YARMOLINSKY 1967). Certainly, the site-specific nature of the curing function, even in phages which lack the homology region, emphasizes the necessity for the SIGNER-BECKWITH model. With regard to exonuclease, whose role is not understood despite extensive characterization of the enzyme (LITTLE 1967), deletion phages show divergent capabilities: cells infected with $i^{\phi 80}h^{+\lambda}$, with ϕtdel 10 or 23 (*cure$^+$rec$^+$*) or ϕtdel 22 (*cure$^-$rec$^+$*) show equal production of phage-induced exonuclease; cells infected with ϕtdel 33 or 1 (*cure$^-$rec$^-$*) show none (G. GUSSIN, personal communication). The localization of genes *cure-integration, exonuclease* and *β-protein*, all in the central region to the left of *susN*, can be considered compatible with what is known of their regulation: subjection to immunity repression and quantitative reduction in phages with a *sus* mutation in gene *N* (GOTTESMAN and YARMOLINSKY 1967; LISIO and WEISSBACH 1965; RADDING and SHREFFLER 1966; SIGNER and BECKWITH 1966; personal observations on the reduced but real ability of *susN$^-$* superinfecting phage to cure). These observations, together with the fact that gene *N* is translated from right to left (HOGNESS, DOERFLER, EGAN and BLACK 1966), hint at the existence of an *N* operon encompassing all of these functions (LIEB 1966).

Described here is a new phage function which promotes the genetic recombination of phage genomes. Its existence was anticipated, since it had been shown that λ shows normal recombination frequencies when vegetative crosses are made in Rec$^-$ bacteria (BROOKS and CLARK 1967; TAKANO 1966; VAN DE PUTTE, ZWENK and RÖRSCH 1966). In mutant phages, phage recombination in a Rec$^-$ host is reduced at least $100 \times$. Although bacterial recombination functions can substitute for that of the phage, preliminary attempts have shown no contribution of the phage to host recombination or UV resistance. The recombination mediated is *not* limited to significance in lysogeny, since its effect was observed within the right and left chromosome arms, independent of the homology region. The ability of λ to supply its own recombination function stands in contrast to its dependence upon the host cell for repair of UV damage (HARM 1963).

The existence of a variety of center-deleted but vegetatively nondefective phages should be useful to further characterization of the center functions of temperate phages.

SUMMARY

A deletion of genetic material from a bacterial lysogen can remove from the prophage one terminal juncture with the bacterial chromosome, without the

elimination of any phage genes essential to phage development. The induction of such a terminally deleted prophage results in the production of rare plaque-forming particles, each with genetic stability and a characteristic DNA content from 100 to 70% of the normal DNA complement of the original ϕ80-λ phage. Measurements of genetic linkage of markers h, c and *deletion* locate the DNA losses at the center of vegetative phage genome, more or less closely linked to h and c and causing genetic shrinkage between h and c. It is this region of the lambdoid chromosome which lies terminally in the prophage, as a result of a circular permutation at lysogenization. It is concluded that when a prophage terminal is deleted, normal prophage excision is prevented, but aberrant excision from within the prophage genome can occur.—A series of deletion phages recovered from a terminal deletion prophage was studied with regard to functional integrity. The ability of deletion plaque-formers to reproduce vegetatively is not impaired, showing that 30% of the ϕ80-λ genome is nonessential to vegetative functions. None of the deletion phages, however, is able to relysogenize, ostensibly because in each case all or part of the region of homology with the bacterial chromosome has been lost. Some deletion phages have lost, in addition, the ability to cure a heteroimmune prophage having the same specificity of attachment to the bacterial chromosome. The genetic determinant of the *curing* function lies near the c end of the central region.—The phages with the longest deletions have lost both the ability to cure and the ability to recombine genetically in a recombination-deficient (Rec⁻) bacterial mutant, though they recombine normally in a homologous Rec⁺ strain. This phage *recombination* function probably maps near h, but possibly near c, or at both locations.

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